



# Complete Genome Segment Sequences of Tomato Chlorotic Spot Virus from Peanut in Haiti

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**ABSTRACT** Tomato chlorotic spot virus (TCSV) is emerging as a significant constraint to vegetable and legume crops in the Americas. The complete genome sequence of a TCSV isolate naturally infecting peanut (*Arachis hypogea*) in Haiti was determined in the effort to build epidemiological knowledge of the virus.

Peanut is an economically important crop cultivated worldwide as a source of dietary protein (1). Several viral species in the genus *Tospovirus*, family *Peribunyaviridae* (2), have been reported to infect peanuts in many countries around the world (3–5). Among them, tomato chlorotic spot virus (TCSV) is receiving increased attention recently due to its expanding geographic and host range in the Caribbean and United States (6–8). Recently, we reported for the first time the occurrence of TCSV in peanut in Haiti (9), which adds to the growing list of tospoviruses infecting peanut. Since this finding has practical implications for the peanut industry in the United States, especially the southeastern and southwestern states, which are the major peanut-producing states, the complete sequences of the small-RNA (S-RNA), medium-RNA (M-RNA), and large-RNA (L-RNA) genome segments of TCSV from peanut were determined by a combination of Sanger sequencing and high-throughput sequencing (HTS). The taxonomic relationship of TCSV from Haiti with TCSV isolates from other countries in the Americas was analyzed to examine the genetic diversity and evolutionary aspects of this emerging tospovirus.

Total RNA was extracted from symptomatic leaves with an RNeasy kit (Qiagen Sciences, Inc., Germantown, MD). The cDNA library preparation was carried out with the TruSeq Stranded Total Ribo-Zero rRNA kit (plant) (Illumina, San Diego, CA) and subjected to HTS on an Illumina HiSeq 2500 platform at the Huntsman Cancer Institute (University of Utah, Salt Lake City, UT). After trimming adapters, raw reads were quality filtered to remove reads with a read length below 125 bp and mapped to the peanut genome (GenBank accession numbers [NC\\_029772](#) and [NC\\_029785](#)) with the default parameters in CLC Genomics Workbench 8.0 (Qiagen Sciences, Inc.). The quality-filtered reads were assembled *de novo* into contigs using the default parameters in CLC Genomics Workbench 8.0. To validate the sequence data from HTS data, individual genomic segments were amplified with segment-specific primers, cloned, sequenced, and assembled as previously described (10).

A total of 44,742,142 quality-filtered reads with an average length of 124.8 bp were obtained. Of these, 2,096,585 reads were mapped to the virus genome, which formed three contigs corresponding to L-RNA, S-RNA, and M-RNA, with an average coverage of 19,730 $\times$ , 16,546 $\times$ , and 5,332 $\times$ , respectively. Comparative sequence analysis showed that the tripartite genome of the TCSV isolate from peanut is 99% identical at the nucleotide level to the reference TCSV isolate from the Dominican Republic (GenBank accession numbers [NC\\_035482](#) to [NC\\_035484](#)). The L-RNA sequence is 8,873 nucleo-

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tides (nt) long and encodes the putative RNA-dependent RNA polymerase (RdRp) in the viral complementary (vc) strand with a predicted molecular weight of 331 kDa. The M-RNA sequence is 4,847 nt long and encodes a 33.94-kDa nonstructural movement protein (NSm) on the viral (v) strand and a 127.68-kDa glycoprotein precursor ( $G_N/G_C$ ) on the vc strand. The S-RNA sequence is 3,311 nt long and encodes the 52.5-kDa nonstructural silencing suppressor (NSs) on the v strand and the 28.5-kDa nucleocapsid protein (N) on the vc strand. The length of the intergenic region in the M-RNA and S-RNA sequences is 346 nt and 886 nt, respectively. The eight conserved nucleotides and complementary sequences present at the 5' (AGAGCAAU) and 3' (AUUGCUCU) termini of the L-RNA, M-RNA, and S-RNA sequences are identical to corresponding sequences in other tospoviruses. The complete genome segment sequences of TCSV from peanut will provide leads for inferring the evolutionary history of the virus and will lead to a better understanding of the extrinsic and intrinsic barriers that influence genome segment exchanges between TCSV and other tospoviruses (7).

**Data availability.** The TCSV genome segment sequences in this announcement are publicly available in GenBank under the following accession numbers: [MH427861](#) (L-RNA), [MH427862](#) (M-RNA), and [MH427863](#) (S-RNA). Raw high-throughput sequencing reads were deposited in the Sequence Read Archive under accession number [SRX5643916](#).

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